

**Four New Species of the Genus *Leptanilla* 1994.
(Hymenoptera: Formicidae) from Spain — Relationships
to Other Species and Ecological Issues**

by

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ABSTRACT

Four new *Leptanilla* species from the Iberian Peninsula and North of Africa are described in this paper: *L. zaballosi*, *L. charonea*, *L. plutonia*, and *L. ortunoi*. Within the Mediterranean species, *L. nana* and *L. revelierei* are related to *L. zaballosi* and *L. charonea*, whilst *L. theryi* is the closer species to *L. plutonia*, and *L. ortunoi*. Some new morphological traits have been successfully used in the comparison of these species: tentorium, madibular setae, last antennal segment, katepisternum, basitarsus of fore legs, shape of femora, and sting. They are proposed as useful traits for the taxonomy of the genus. Other controversial traits (such as head spots and tibial spurs) are studied, and their taxonomical relevance is analyzed. A morphometrical Cluster Analysis performed with measurements from most of the known worker-based *Leptanilla* species showed 3 groups of species. These groups include small, medium and large sized species. There is no kind of geographical grouping in this ordination. Finally, the large quantity of specimens found and the high frequency of these findings is extensively discussed in the context of the collection methods employed and the biotopes surveyed, which are not common in entomological surveys.

Keywords: *Leptanilla*, new species, taxonomy, biotopes, collection methods, Iberian Peninsula

INTRODUCTION

The genus *Leptanilla* Emery is composed of minute and hypogaecic ants. The scarcity of specimens found throughout its history has led to a comparative ignorance about many questions on these ants, such as their phylogenetic relationships, their biology, and the taxonomic kinship between individuals from different castes (workers, females, and males). Some recent studies have cast new light on these questions. Baroni Urbani (1977) and Bolton (1990) have revised the taxonomy and higher classification of the whole subfamily Leptanillinae

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Emery, and Masuko (1990) has offered a fine and detailed study on the behavior, biology, and ecology of cultured colonies of *L. japonica* Baroni Urbani.

To date, only 5 species described from workers (others have only been described for males) are known from the Mediterranean region: *L. vaucheri* Emery, *L. theryi* Forel, *L. nana* Santschi, *L. revelierei* Emery, and *L. judaica* Kugler. With the exception of the latter, a recent discovery (Kugler 1986), they were described almost a century ago. Females are only known for 2 of them (*L. theryi* and *L. revelierei*). On the Iberian peninsula there are only 2 previous recordings, both of single specimens, of this genus: a male collected in the Jaen province named as '*Leptanilla* sp. B' (Baroni Urbani 1977) and a worker identified as *L. revelierei* (Tinaut 1987).

In this paper we describe 4 new species of the genus *Leptanilla* from the Mediterranean region, 1 of them with female. Three are from the Iberian Peninsula, and the fourth is from Ceuta (North Africa). An unusually large number of specimens was found. This could be explained by the collection method and the biotopes sampled, which are not common in entomological surveys. We compared the new species with those most related *Leptanilla* species from the Mediterranean region, using classical as well as new morphological traits. A quantitative morphological comparative view of the workers of the majority of known *Leptanilla* species from all over the world is also provided.

MATERIALS AND METHODS

Most of the *Leptanilla* specimens of these new species were collected by means of a method employed by some coleopterologists studying hypogaecic beetles, almost always in the same type of biotope. The method simply consisted of taking very large soil samples (mainly composed of moist sand with a low organic content) from slopes of small seasonal water courses. These slopes did not exceed a height of 2m, were abrupt, and suffered heavy water erosion, as they lacked a surface layer of organic matter. The samples were taken at a medium height on the slope, previously removing an external layer of soil. Sandbags of about 40kg of material were taken from each slope, and these were taken directly to the laboratory or previously put in water for the deposition of the sand and the collection of remains of organic matter and insects with a sieve ('lavage de terre' method; Normand 1911; Coiffait 1958; Zaballos 1990). The samples (filtered or not) were distributed afterwards in different Berlese-Tullgren devices and left during several days for their desiccation and the extraction of the insects.

All specimens were cleaned by immersion in a 100% solution of lactic acid for a period of 48-72h. They were studied in ephemeral preparations of lactic acid on microscope excavated slides and in permanent preparations of Hoyer on normal slides. Some were preserved in these latter preparations, while others were preserved in Scheerpeltz solutions. We strongly recommend these (or similar) procedures for handling and preserving *Leptanilla* specimens. If they are dry-mounted, they can be easily broken due to their extreme fragility, and many of their morphological traits cannot be studied. These are only noticeable by microscopic observation because of their minute size or because they must be observed by transparence (e.g., endoskeleton). All the collection data related to the specimens of the new species are given in the following section.

We compared our species with type material from the Museo Civico di Storia Naturale of Genova and the Naturhistorisches Museum of Basel. This material includes types, paratypes, and other specimens of the following taxa: *L. japonica* Baroni Urbani (workers), *L. kubotai* Baroni Urbani (workers), *L. nana* Santschi (workers), *L. revelierei* Emery (workers and female), *L. revelierei* var. *bimaculata* Emery (workers), *L. revelierei* var. *sardoa* Emery (workers), *L. revelierei* ssp. *chobauti* Emery (workers), and *L. theryi* Forel (workers and female). It must be stressed that many of the type specimens studied are not preserved in a fit state, because they were dry-mounted or not adequately prepared on slides, with several parts of the body broken or deformed. Unless the specimens are properly prepared, the traits of internal anatomy cannot always be appreciated, and the observation of the smallest external traits is difficult.

In addition to the classical traits in the taxonomy of the genus (mandibular teeth, scapes, tibial spurs, shapes of head, alitrunk, and petiolar nodes, etc.), we studied several new traits that proved to be successful in the discrimination of the different species: tentorium, mandibular setae, last antennal segment, katapisternum, basitarsus of fore legs, shape of femora, and sting.

The diagrams were drawn using a Zeiss binocular microscope. Micrographs were taken with a Jenaual Zeiss photomicroscope equipped with planechromatic object lenses.

In order to morphometrically compare both the new species described in this work and other known species, whose measurements are offered by Baroni Urbani (1977), we made the same measurements and calculated the same indexes employed by this author. Abbreviations and magnifications are indicated in parenthesis. All measurements were made using an Izumi microscope, with a magnification of 200x

(error = $\pm 0.01\text{mm}$) or 100x (error = $\pm 0.02\text{mm}$). Measurements and indexes are defined below.

Cephalic length (CL) (200x): Length of the head in dorsal view, measured between clypeus and occiput along the sagittal plane.

Cephalic width (CW) (200x): Maximum width of head in dorsal view.

Scape length (SL) (200x): Length of scape in dorsal view, excluding the articular condilus.

Petiole length (PL) (200x): Length of petiolar node in dorsal view along the sagittal plane, excluding the petiolar stem.

Petiole width (PW) (200x): Maximum width of petiolar node in dorsal view.

Postpetiole length (PPL) (200x): Length of postpetiolar node in dorsal view along the sagittal plane.

Postpetiole width (PPW) (200x): Maximum width of postpetiolar node in dorsal view.

Total length (TL) (100x): Length of the whole individual, measured between anterior margin of head and tip of gaster, along the sagittal plane.

Cephalic index (CI) = $CW / CL \cdot 100$

Scape index (SI) = $CW / SL \cdot 100$

Petiole index (PI) = $PW / PL \cdot 100$

Postpetiole index (PPI) = $PPW / PPL \cdot 100$

Petiole-postpetiole index (PPPI) = $PW / PPW \cdot 100$

The morphological affinities of the 4 new species with the other *Leptanilla* worker-based species, as well as the ordination of all of them, according to these quantitative data, were studied by means of a Cluster Analysis with sequential agglomerative grouping ($\alpha = 0.625$; $\beta = -0.25$) (Sokal & Sneath 1963; Lance & Williams 1967a, b; Wilkinson 1987). This analysis was performed using minimum and maximum values of all measurements and indexes, since this was the information available for all *Leptanilla* species from the work of Baroni Urbani (1977).

In order to compare 2 of the new species with *L. revelierei* using morphometrical data, a Discriminant Analysis (Jennrich & Sampson 1983; Wilkinson 1987) was performed on these three species. All measurements indicated above were made in 30 randomly selected specimens of *L. zaballosi* n. sp., all specimens of *L. charonea* n. sp. that were in a fit state (33), and the following type material: 12 specimens of *L. revelierei* ssp. *chobauti*, 3 of *L. revelierei* var. *sardoa*, and 1 of *L. revelierei* var. *bimaculata*.

RESULTS

Description of the new species:

Except as noted, all specimens were collected using the collection method described in the previous section. The known geographical distribution of the new species is shown in Fig. 14. All morphological traits mentioned in the descriptions have been examined in all the specimens of the new species. The extent of the variability of some of them has been taken into account in these descriptions and in the comparisons among species.

***Leptanilla zaballosi* n. sp.**

Worker (Figs. 1 & 11). Colour pale yellow, slightly orange-coloured. Legs paler. Margins of alitrunk, petiole, and postpetiole brownish in dorsal and lateral view. Integument very feebly striated on the anterior margins of pronotum and mesonotum, and ventrally on the alitrunk and petiolar node. Pilosity short, scattered, subdecumbent to decumbent in the antennae and tarsi, and erect to suberect on the rest of the body; more scarce on the alitrunk.

Head elongated, flattened above, wider than alitrunk, with subparallel sides. Occiput straight or feebly emarginated. Tentorium with two short divergent arms directed backwards and two long divergent arms directed forwards. The anterior arms are U-shaped. In their mid-part they bear a pair of external, tooth-like protuberances and another pair of internal, roundish plates. Clypeus straight, neither notched nor protruding. Mandibles long and narrow, with 4 teeth: a long apical tooth, two large grouped sharp-pointed basal teeth, and a tiny median tooth placed in the inner part of the mandible, beside the basal teeth. Internal side of mandibles with 3 (usually) or more long setae. Antennae stout, with 12 segments. Scapes slender, distally swollen, reaching the middle of the head, as long as the 5 proximal funicular segments together. First and second funicular segments pedunculate. The first one as long as the 2nd and 3rd together. Funicular segments 2 - 6 wider than long. Segments 7 - 10 quadrate, as wide as long. Last funicular segment elongated, slightly longer than the two preceding segments together, with a sharp-pointed tip.

Alitrunk elongated, but clearly shorter and narrower than the gaster. Prothorax subovoidal, almost as wide as long in dorsal view, separated by a feebly promesonotal furrow from the rest of the alitrunk. Dorsal margin of the pronotum softly convex in lateral view. Prosternum feebly protruded. Mesepinotum also subovoidal, but clearly longer than wide; its dorsal margin is straight in lateral view without depression between

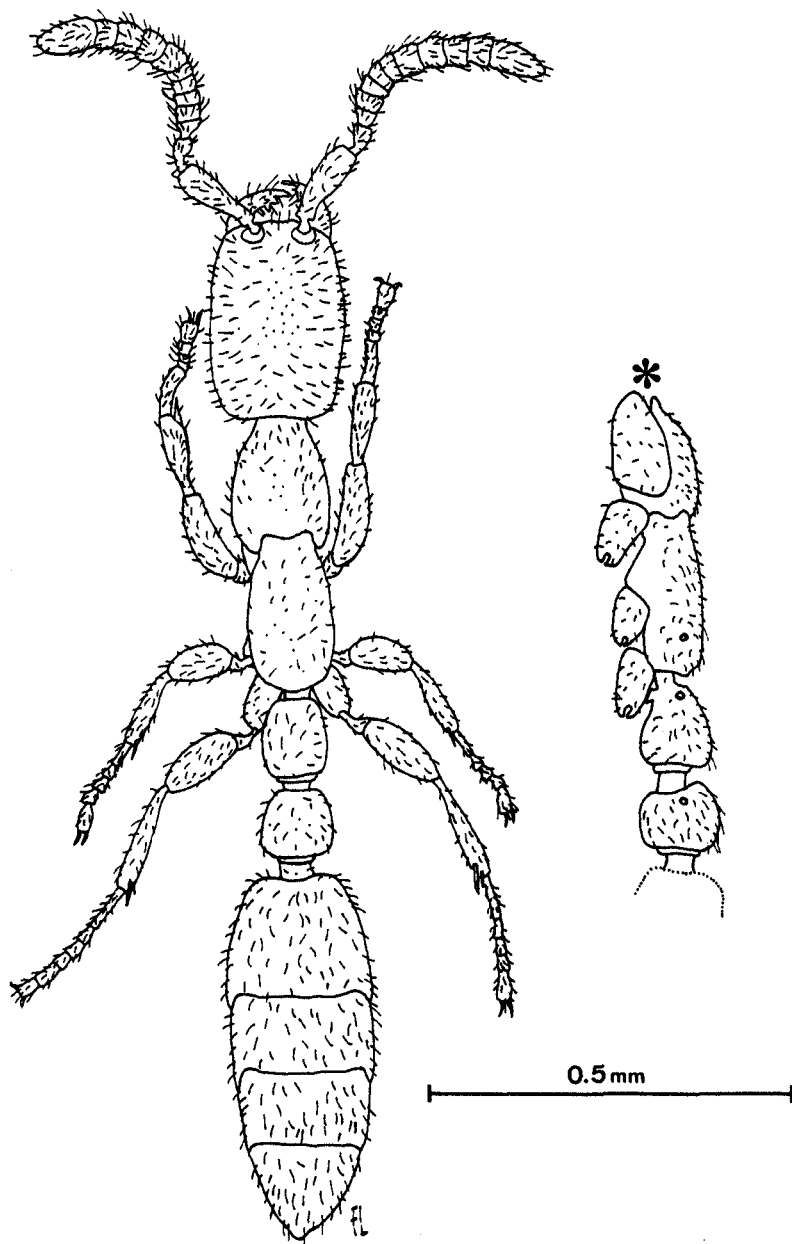


Fig. 1. *Leptanilla zaballosin*, sp. Holotype worker. Left: dorsal view. Right: lateral view of thorax and petiolar nodes. The asterisk indicates a small disjoining between the sclerites of the prothorax.

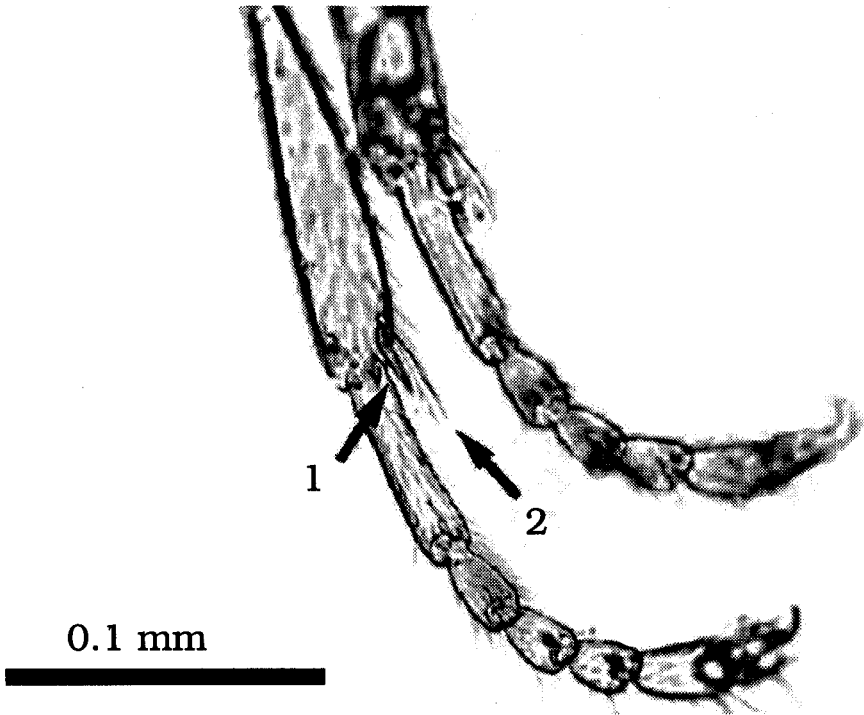


Fig. 2. Lateral view of hind legs of *Leptanilla zaballosi* n. sp. showing the two tibial spurs. 1: Small, simple spur. 2: large, pectinated spur (out of focus).

the mesonotum and the propodeum. Katepisternum protruded. Hind dorsal margin of propodeum rounded far behind the propodeal spiracle. Legs stout; fore tibiae and all femora swollen. Basitarsus of fore legs with a projection on its ventral anterior margin. Mid tibiae with a small, simple spur. Hind tibiae with this same spur, plus a second one, large and pectinated (Fig. 2).

In dorsal view, both petiolar and postpetiolar nodes subquadrangular, clearly wider posteriorly; their anterior margin sometimes feebly emarginated. Petiolar node longer than wide. Postpetiolar node approximately as long as wide. In lateral view, both nodes dorsally and ventrally convex. Petiolar spiracles clearly visible. Dorsal anterior margin of the petiolar node rounded and directed backwards, ventrally with a tooth-like keel followed by several little teeth of decreasing size. Postpetiolar node clearly emarginated in its anterior margin, without any projection in its ventral margin.

Gaster ovoidal, anterior margin emarginated. First gaster tergite longer than the others. Sting long, not protruding, with the bulb wider

than the shaft.

Minimum and maximum of measurements (in mm) and indexes.

CL = 0.22 - 0.28; CW = 0.17 - 0.20; SL = 0.09 - 0.12; PL = 0.09 - 0.11; PW = 0.06 - 0.10; PPL = 0.07 - 0.10; PPW = 0.09 - 0.11; TL = 1.21 - 1.55; CI = 60.87 - 83.33; SI = 140.00 - 214.28; PI = 71.43 - 100.00; PPI = 100.00 - 128.57; PPPI = 71.43 - 114.29 (N = 229).

Holotype. Worker from Bohonal de Ibor, Cáceres, SPAIN, UTM 30STK80, altitude = 350m, 9-V-89, J.P. Zaballos leg.

Paratypes. (1) 83 workers from the same sample as the holotype. (2) 5 workers from Casa de Campo, Madrid, Spain, UTM 30TVK37, altitude = 660m, 13-II-1987, L.G. Gómez and C.F. Hernan leg. (3) 24 workers from Candeleda, Avila, Spain, UTM 30TUK04, altitude = 250m, 9-V-1988, J.P. Zaballos leg. (4) 66 workers from Navas de Estena, Ciudad Real, Spain, UTM 30SUJ77, altitude = 700m, 10-V-1988, V. Ortuño leg. (5) 59 workers from Caserío Cuaternos, Cáceres, Spain, UTM 30TTK73, altitude = 250m, 17-V-1988, J.P. Zaballos leg. (6) 1 worker from Vega de Mesillas, Cáceres, Spain, UTM 30TTK73, altitude = 275m, 17-V-1988, J.P. Zaballos leg. (7) 50 workers from Valencia de Alcántara, Cáceres, Spain, UTM 29SPD56, altitude = 500m, 23-III-1990, J.P.

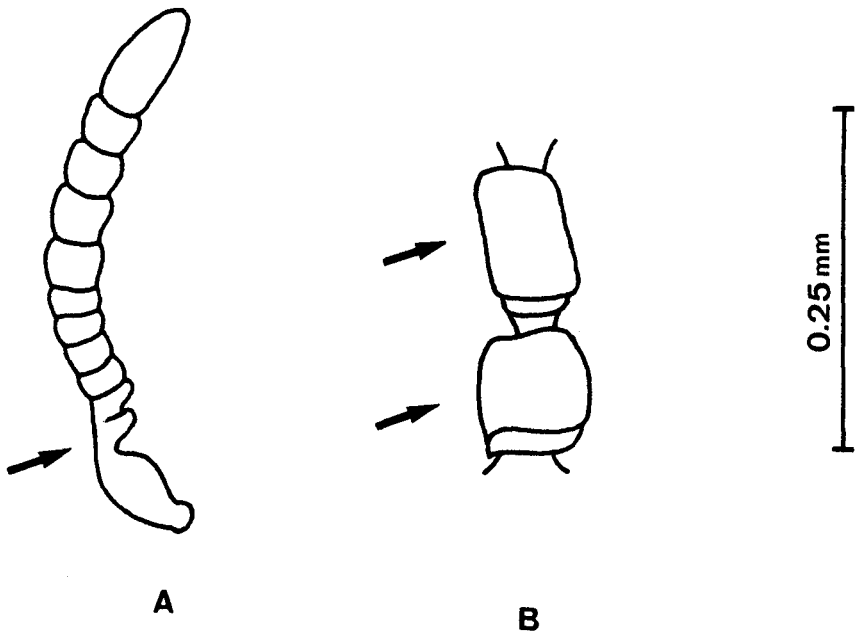


Fig. 3. Teratologies of two specimens of *Leptanilla zaballosi* n. sp. A: Antenna with sinfisocery. B: Deformed petiolar nodes. Both in dorsal view. Piloosity omitted.

Zaballos leg. (8) 67 workers from San Martin de Valdeiglesias, Madrid, Spain, UTM 30TUK87, altitude = 670m, 9- XII - 1991, soil sample from a small irrigation ditch, J.C. Atienza leg. (9) 32 workers from Membrio, Cáceres, Spain, UTM 29SPD56, altitude = 350m, 18 - XII - 1991, J.P. Zaballos leg.

The holotype and 372 paratypes are preserved in the Cátedra de Entomología collection, Facultad de Biología, Universidad Complutense de Madrid. Three paratypes are preserved in each collection of the following institutions or persons: Naturhistorisches Museum (Basel), Museo Civico di Storia Naturale (Genova), Museum of Comparative Zoology (Harvard), Dr. J. A. Tinaut (Universidad de Granada), Dr. X. Espadaler (Universidad Autònoma de Barcelona).

Derivatio nominis. This species is dedicated to Dr. Juan P. Zaballos, who collected many of the specimens and gave us useful information about the captures.

Observations. Two teratological specimens were found in the material studied. The first one was found in the sample from Navas de Estena. It is a case of synfisocery (Balazuc 1948), having the two first funicular segments fused with the scape (Fig. 3A). The basal constrictions of both segments are visible. The scape is abnormally short. The second teratological case was found in the sample from Caserio Cuaternos. The petiolar and postpetiolar nodes of the specimen are deformed (Fig. 3B), with nearly parallel sides not widened posteriorly.

Several specimens from three different samples have dark red spots near the anterior margin of head. They correspond to masses of internal tissues of the head, which are seemingly the mandibular muscles. They usually appear as a pair of circular spots placed on both sides of head but are highly variable among individuals, even within the same sample, as may be seen in Fig. 4. As commented below, these spots also appear in other species. This trait was the only discriminant feature employed by Emery (1899) to describe the *bimaculata* variety of *L. revelierei*. Baroni Urbani (1977) reported that in the type specimens of this variety the spots are also highly variable and, consequently, he did not consider *bimaculata* as a valid taxon. We studied 2 type specimens of this variety and we have found that the spots do not differ at all from those of our specimens. We thus regard this trait to lack any taxonomic usefulness and we completely agree with the opinion of Dr. Baroni Urbani on the variety *bimaculata*.

***Leptanilla charonea* n. sp.**

Worker (Figs. 5 & 11). Colour pale yellow, clearer than *L. zaballosi*. Legs paler. Margins of alitrunk, petiole, and postpetiole brownish in

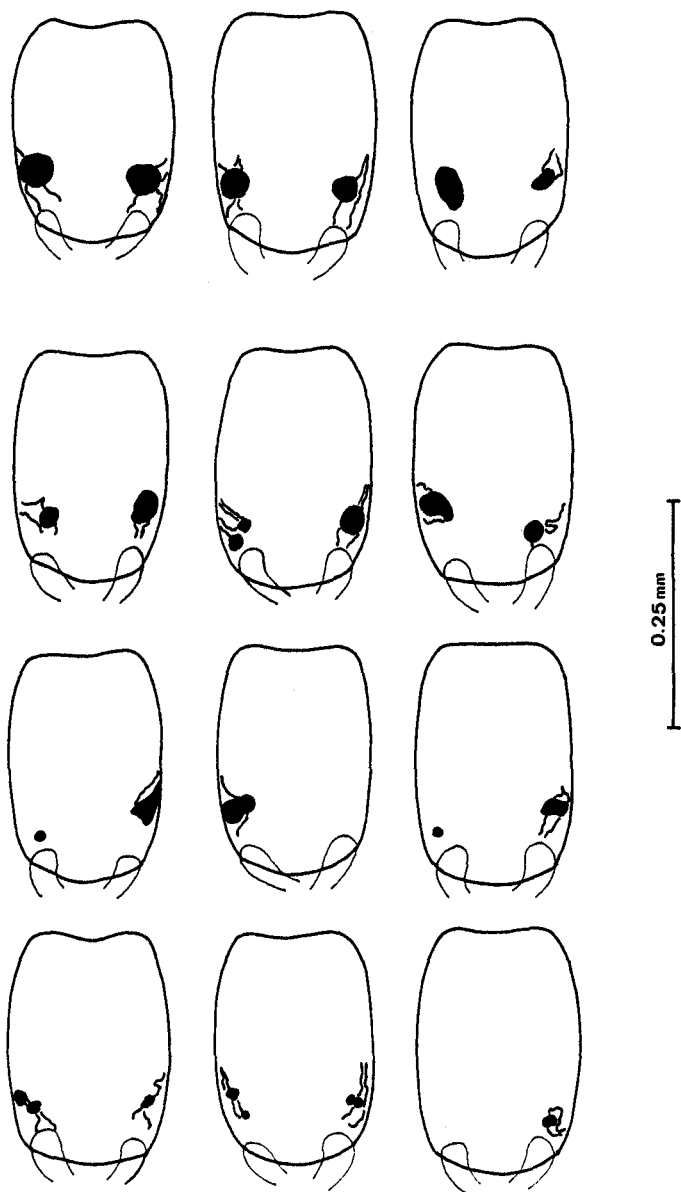


Fig. 4. Series of specimens of *Leptanilla zaballosin*, sp. showing a decreasing spread of the red spots on the anterior margin of the head (dorsal view). Pilosity omitted.

dorsal and lateral view. Integument feebly striated on the anterior margins of pronotum and mesonotum, and ventrally on the alitrunk and petiolar node. Pilosity shorter and more scattered than in *L. zaballosi*, subdecumbent to decumbent in the antennae and tarsi, and erect to suberect on the rest of the body, more scarce on the alitrunk.

Head elongated, flattened above, wider than alitrunk, with sides more parallel than in *L. zaballosi*. Occiput straight. Tentorium (Fig. 6) with general morphology as in *L. zaballosi* but with the following differences: anterior arms U- or V-shaped, tooth-like protuberance less protruding, internal plates with a straight anterior margin. No spots on the head. Clypeus straight, neither notched nor protruding. Mandibles long and narrow, with 4 teeth: a long apical tooth, 2 grouped, not sharp-pointed basal teeth, and a minute, rounded median tooth placed on the inner part of the mandible, besides the basal teeth. Internal side of mandibles with 2 long setae. Morphology of antennae as in *L. zaballosi*, but with the following differences: scapes swollen, clearly shorter and wider, not reaching the middle of the head. Last funicular segment shorter (as long as the 2 preceding segments together) with a more rounded tip.

Alitrunk elongated, but clearly shorter and narrower than the gaster. Prothorax subovoidal, less long than in *L. zaballosi*, separated by a feebly promesonotal furrow from the rest of the alitrunk. In lateral view, dorsal margin of the pronotum not so convex as in *L. zaballosi*, with the same height as the mesepinotum. Prosternum and katepisternum less protruding than in *L. zaballosi*. Mesepinotum also subovoidal, but clearly longer than wide, with sides more convex than in *L. zaballosi*; its dorsal margin is straight in lateral view, without depression between the mesonotum and the propodeum. Hind dorsal margin of propodeum rounded far behind the propodeal spiracle. Morphology of legs (including the tibial spurs) as in *L. zaballosi*, but with the following differences: femora shorter, more stout; projection of the basitarsus of fore legs longer.

Morphology of petiolar nodes as in *L. zaballosi*, but with the following differences: in dorsal view, both petiolar and postpetiolar nodes wide posteriorly, but less clearly than in *L. zaballosi*. In lateral view, dorsal anterior margin of the petiolar node acute and not directed downwards; petiolar tooth-like keel shorter, without posterior teeth behind it.

Morphology of gaster as in *L. zaballosi*, but with more parallel sides and the sting bulb not as wide in comparison with the shaft.

Female (Fig. 7). Ergatoid, dichthadiiform. In physogastric state.

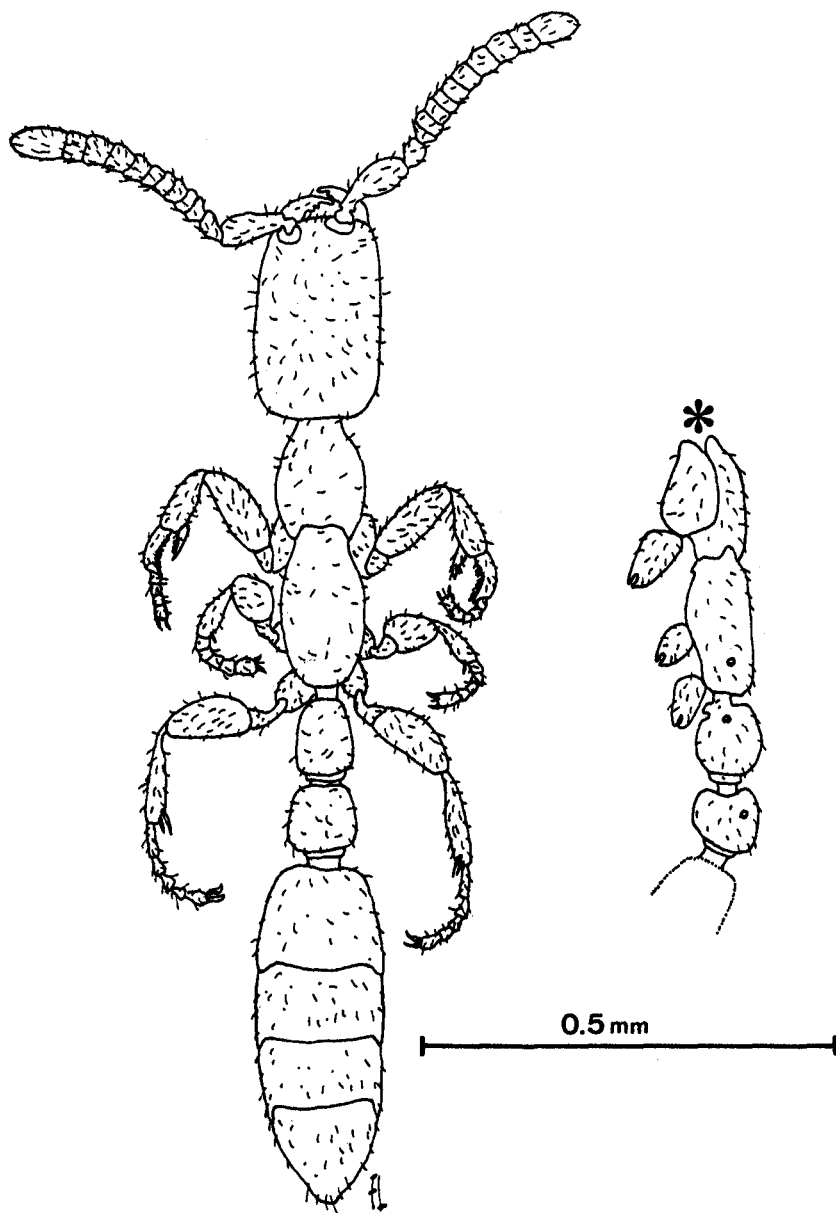


Fig. 5. *Leptanilla charonean* n. sp. Holotype worker. Left: dorsal view. Right: lateral view of thorax and petiolar nodes. The asterisk indicates a small disjoining between the sclerites of the prothorax.

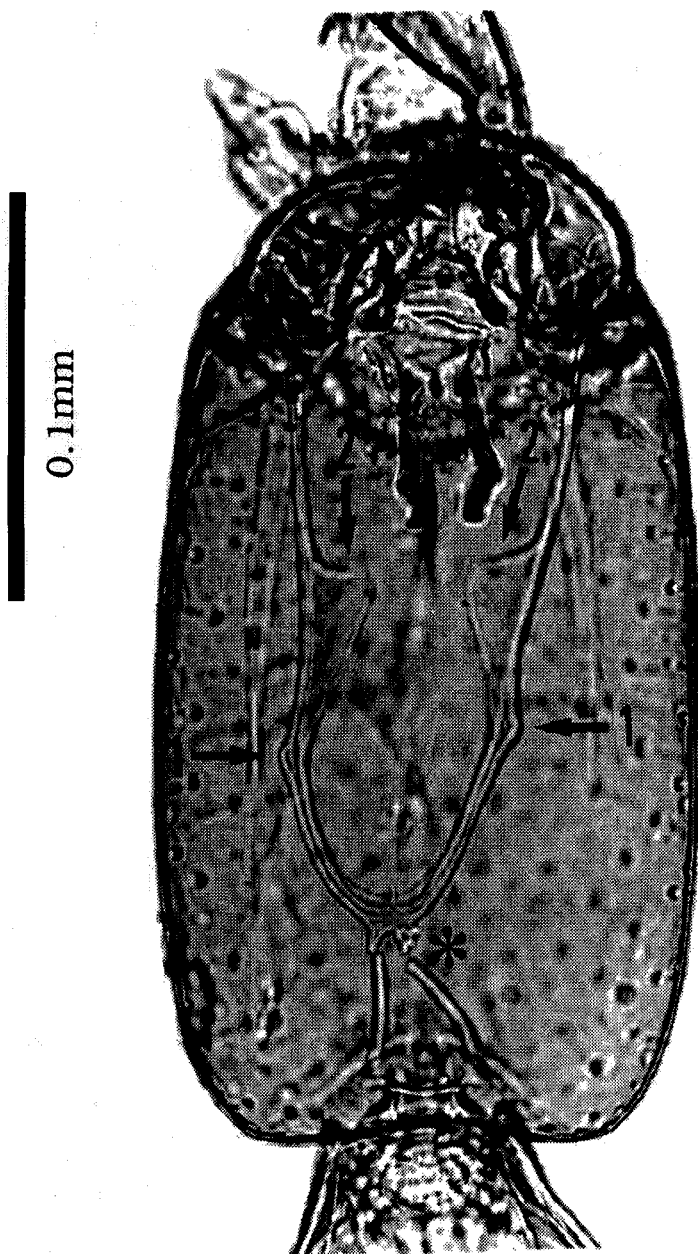


Fig. 6. Head of *Leptanilla charonean*, sp. in dorsal view showing the tentorium through the exoskeleton of the head. 1: External, tooth-like protuberances. 2: Internal, roundish plates. Asterisk indicates a break in the posterior arms of the tentorium.

Colour, sculpture and pilosity similar to the worker, but with the following differences: integument margins not brownish-coloured, pilosity slightly more abundant, gastral hairs very long.

Head elongated, flattened above, not wider than the alitrunk, with sides more parallel than in the worker. Occiput straight. Tentorium as in the worker, but with roundish internal plates. No spots on the head. Clypeus with a small central roundish protuberance. It bears 3 long setae (1 central and 2 lateral) on its anterior margin, almost reaching the inner side of mandibles. Mandibles falcate, long and narrow, ending in a short masticatory margin without teeth (excluding an apical sharp-pointed tip that could be considered as an apical tooth). Internal side of mandibles with 5-6 long setae. Morphology of antennae as in the worker, but the last funicular segment with a sharp-pointed tip.

Alitrunk elongated, with sides more parallel and more flattened above than in the worker. Prothorax subquadrangular, almost as wide as long, separated by a strong promesonotal furrow from the rest of the alitrunk. Mesepinotum subovoidal, but clearly longer than wide. In lateral view, dorsal margins of pronotum and mesepinotum straight. Hind dorsal margin of propodeum acute. Morphology of legs as in the worker, with clearly swollen femora.

Petiole one-segmented. Petiolar node quadrangular, as long as wide, slightly emarginated posteriorly. In lateral view, dorsal margin straight; anterior part of the ventral margin with a small protuberance.

Gaster spherical (physogastric), with granulate surface, due to the ovarian development; far longer and wider than the rest of the body. Tergite of the first gastral segment subquadrangular, clearly emarginated anteriorly, and widening posteriorly. The other gastral tergites have different shapes but are always more or less rectangular.

Minimum and maximum of measurements (in mm) and indexes.

Workers (N = 33): CL = 0.21 - 0.26; CW = 0.14 - 0.17; SL = 0.07 - 0.10; PL = 0.07 - 0.10; PW = 0.06 - 0.09; PPL = 0.07 - 0.09; PPW = 0.07 - 0.09; TL = 1.07 - 1.24; CI = 54.99 - 77.78; SI = 137.50 - 233.33; PI = 71.43 - 116.67; PPI = 100.00 - 116.67; PPPI = 71.43 - 116.67. Female (N = 1): CL = 0.29; CW = 0.19; SL = 0.09; PL = 0.12; PW = 0.13; PPL = 0.14; PPW = 0.20; TL = 1.31; CI = 65.51; SI = 211.11; PI = 108.33; PPI = 142.86; PPPI = 65.00.

Holotype. Worker from Mejordada del Campo, Madrid, Spain, UTM 30TVK57, 3-VI-1988, J.M. Barandica leg.

Gynotype. Female from the same sample as the holotype.

Paratypes. (1) 32 workers from the same sample as the holotype. (2) 3 workers from Casa de Campo, Madrid, Spain, UTM 30TVK37, altitude

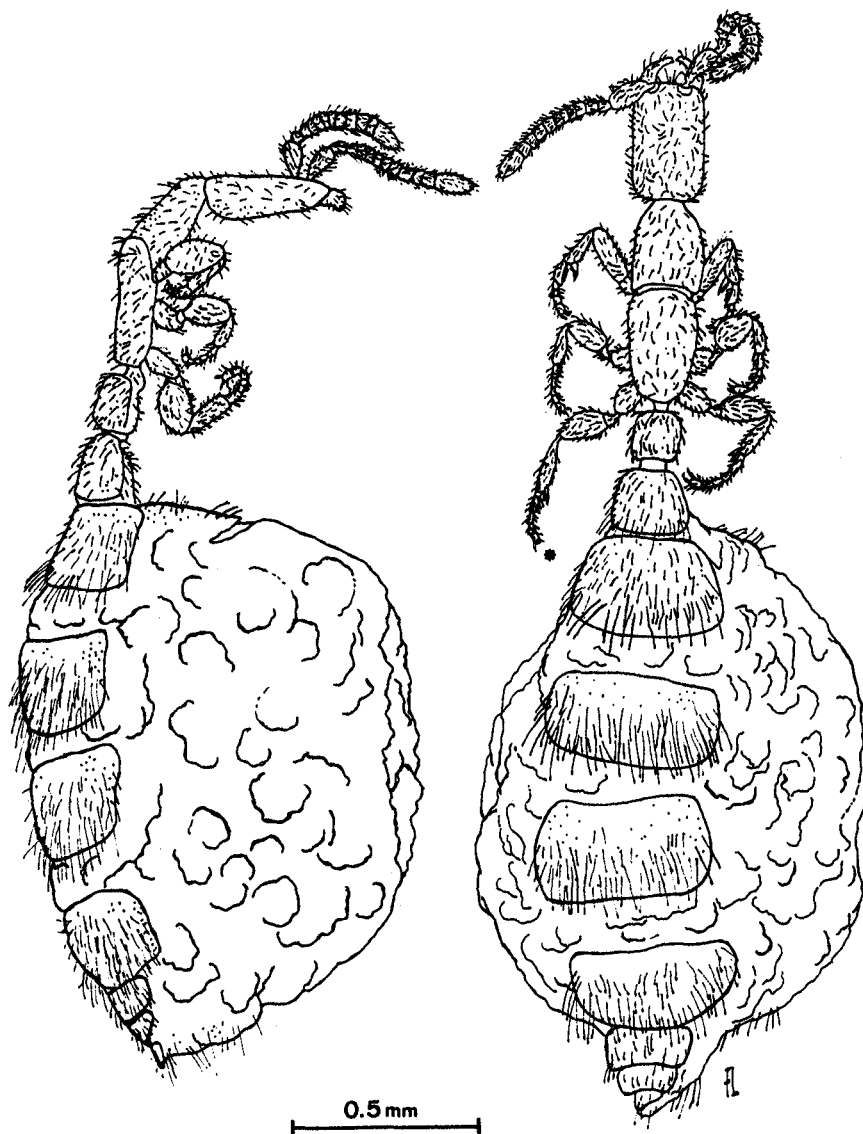


Fig. 7. *Leptanilla charonean* sp. Physogastric female (gynetype) in lateral (left) and dorsal (right) view. The asterisk indicates the lack of one last tarsal segment.

= 660m, 13-II-1987, L.G. Gómez and C.F. Hernan leg. (3) 4 workers from La Higuera, Avila, Spain, UTM 30TUK25, altitude = 425m, 2-VI-1988, J.P. Zaballos leg.

The holotype, the gynotype and 34 paratypes are preserved in the Cátedra de Entomología collection, Facultad de Biología, Universidad Complutense de Madrid. One paratype is preserved in each collection of the following institutions or persons: Naturhistorisches Museum (Basel), Museo Civico di Storia Naturale (Genova), Museum of Comparative Zoology (Harvard), Dr. J. A. Tinaut (Universidad de Granada), Dr. X. Espadaler (Universidad Autónoma de Barcelona).

Derivatio nominis. The specific name *charonea* is derived from the latin term *charon*, *charontis*, name of the boatman that took the souls of the dead towards the inside of the kingdom of Hades.

Observations. The sample from Mejorada del Campo containing a female was collected by direct excavation while digging a nest of *Leptothorax fuentetSantschi*. The excavation site was on the upper part of the slope of a small dry rivulet. The soil was very moist and covered with a layer of moss. A small ovoidal chamber (length: 6mm, diameter: 2mm) was found under a small root at a depth of 13cm, just 1.5cm under the lower limit of the *Leptothorax* nest. A small gallery departing from this chamber and directed downwards could be followed for 6mm. The physogastric female, isolated, was found 1cm below. A soil sample from around the nest was taken and put in a Berlese-Tullgren device. Four more workers were obtained by this method. All living specimens were taken to the laboratory and installed in a plastic tube nest with a water reservoir separated by a small piece of cottonwool. The ants moved to beside the cottonwool. Honey mixed with sand and small insects were given to the ants. After 2 days of laboratory rearing the colony was infested by fungi and had to be fixed in Scheerpeltz to avoid the destruction of the specimens.

The queen physogastry seems to indicate that this colony was collected just at the beginning of the 'egg-laying stage' (Masuko 1990).

***Leptanilla plutonia* n. sp.**

Worker (Figs. 8 & 11). Colour yellow orange-coloured. Legs paler. Margins of head, alitrunk, petiole, postpetiole, and first gastral segment brownish in dorsal and lateral view. Integument striated (much more clearly than in *L. zaballoi* and *L. charonea*) in the anterior margins of pronotum and mesonotum, and ventrally in the alitrunk and petiolar node. Pilosity longer and less scattered than in *L. zaballoi* and *L. charonea*, subdecumbent to decumbent in the antennae and tarsi, and erect to suberect in the rest of the body; more scarce in the alitrunk.

Head flattened above, wider than alitrunk, less elongated and with less parallel sides than in *L. zaballoi* and *L. charonea*. Occiput

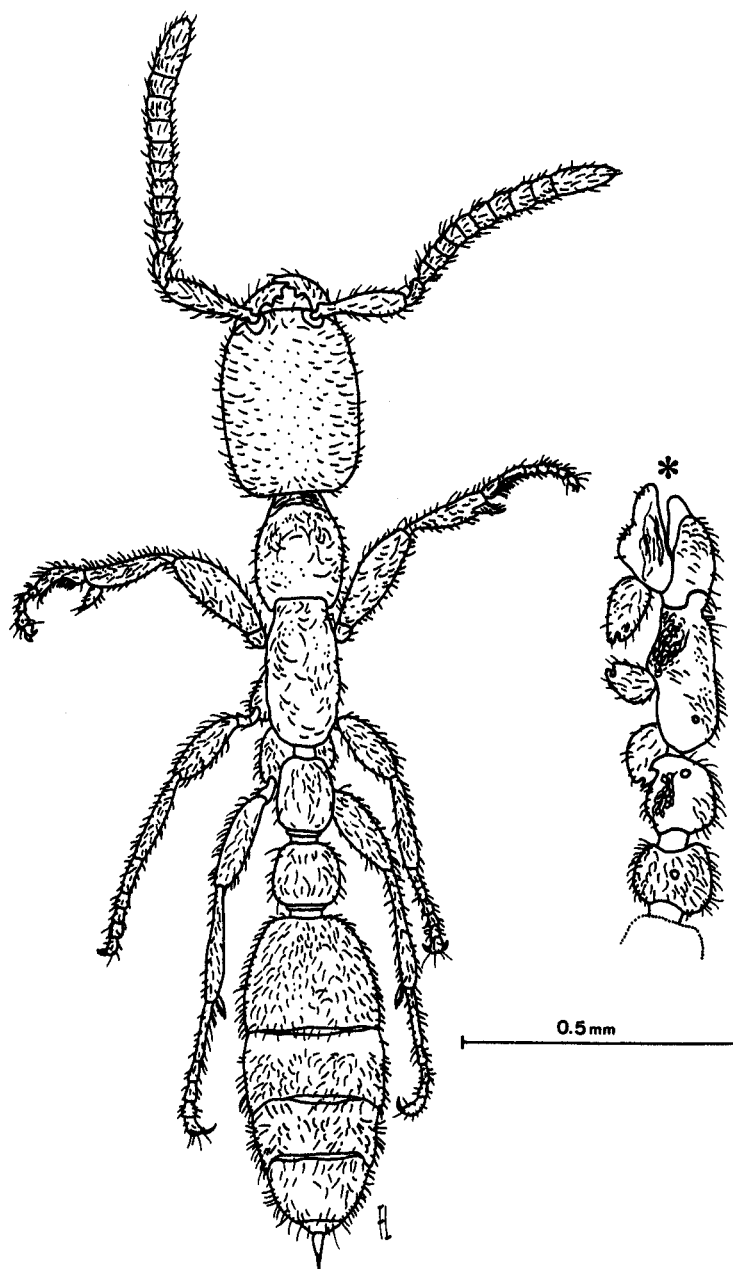


Fig. 8. *Leptanilla plutonia* n. sp. Holotype worker. Left: dorsal view. Right: lateral view of thorax and petiolar nodes. The asterisk indicates a small disjoining between the sclerites of the prothorax.

straight. Tentorium with general morphology as in *L. zaballosi*, but bigger and with more elongated internal plates. Two small dark red spots inside the head in the right anterior margin. Clypeus straight, neither notched, nor protuding. Mandibles long and narrow, with 4 teeth: a long apical tooth, 2 large grouped sharp-pointed basal teeth, and a small median tooth placed in the margin of the mandibles besides the basal teeth. Internal side of mandibles with 3 long setae. Antennae with 12 segments, more stout and with scapes more slender than in *L. zaballosi* and *L. charonea*. The latter reach the middle of the head, and they are as long as the 4 proximal funicular segments together. First and second funicular segments pedunculate. The first one not as long as the 2nd and the 3rd together. Funicular segments 3-10 quadrate, forming 2 groups of different size: 3-6 (smaller) and 7-10 (bigger). Last funicular segment elongated, clearly longer than the two preceding segments together, with a sharp-pointed tip.

Alitrunk elongated, but clearly shorter and narrower than the gaster. Prothorax subovoidal, almost as wide as long in dorsal view, separated with a deep promesonotal furrow from the rest of the alitrunk. Prosternum strongly protruded. In lateral view, pronotum more convex than in *L. zaballosi* and *L. charonea*. Mesepinotum also subovoidal, but clearly longer than wide; its dorsal margin is straight in lateral view, without depression between the mesonotum and the propodeum. Katepisternum protruded. Hind dorsal margin of propodeum rounded (more softly than in *L. zaballosi* and *L. charonea*) far behind the propodeal spiracle. Legs stout, but fore tibiae and all femora are not swollen as in *L. zaballosi* and *L. charonea*. Basitarsus of fore legs without projection on its ventral anterior margin. Tibial spurs as in *L. zaballosi*.

In dorsal view, petiole node elongated, clearly longer than wide, with its anterior margin emarginated. Postpetiolar node subquadrangular, as long as wide, not as clearly wider posteriorly as in *L. zaballosi* and *L. charonea*. Its anterior margin feebly emarginate. In lateral view, both nodes dorsally and ventrally convex. Petiolar spiracles clearly visible. Dorsal anterior margin of the petiolar node rounded, slightly directed downwards, ventrally with a plate-like keel feebly serrated, followed by three minute teeth. Postpetiolar node clearly emarginated in its anterior margin, without any projection in its ventral margin.

Gaster ovoidal, less slender than in *L. zaballosi* and *L. charonea*; anterior margin feebly emarginated. First gaster tergite longer than the others. Sting comparatively shorter than in *L. zaballosi* and *L. charonea*, protuding, with the bulb clearly wider than the shaft.

Measurements (in mm) and indexes. CL = 0.32; CW = 0.25; SL =

0.14; PL = 0.14; PW = 0.11; PPL = 0.12; PPW = 0.12; TL = 1.74; CI = 76.92; SI = 181.82; PI = 81.82; PPI = 100.00; PPPI = 90.00 (N = 1).

Holotype. Worker from Navas de Estena, Ciudad Real, Spain, UTM 30SUJ77, altitude = 690m, 10-V-1988, V. Ortuño leg. Preserved in the Cátedra de Entomología collection, Facultad de Biología, Universidad Complutense de Madrid.

Derivatio nominis. The specific name *plutonia* is derived from the latin term *pluto*, *plutonis*, by-name of Hades, god of the subterranean world in Greek and Latin cultures.

***Leptanilla ortunoi* n. sp.**

Worker (Figs. 9 & 11). Colour yellow orange-coloured. Legs paler. Margins of head, alitrunk, petiole, and postpetiole brownish in dorsal and lateral view. Integument striated (much more clearly than in *L. zaballosi* and *L. charonea*) in the anterior margins of pronotum and mesonotum, and ventrally in the alitrunk and petiolar node. Pilosity as in *L. plutonia*, but more scarce.

Head flattened above, wider than alitrunk; shape as in *L. plutonia*. Occiput straight. Tentorium with general morphology as in *L. zaballosi*, but bigger and with shorter and more parallel anterior arms. Tooth-like protuberances more protuding and internal plates shorter and more roundish than in *L. plutonia*. One small dark red spot inside the head on the right anterior margin. Clypeus straight, neither notched, nor protuding. Mandibles slightly more stout than in the other three species, with 4 teeth: a long apical tooth, 2 large grouped sharp-pointed basal teeth, and a small median tooth placed on the margin of the mandibles besides the basal teeth. Internal side of mandibles with 4 long setae. Antennae with 12 segments; their general morphology as in *L. plutonia*, although both antennae lack several terminal segments.

Alitrunk elongated, but clearly shorter and narrower than the gaster. Prothorax subovoidal, shorter than in *L. plutonia*, separated by a deep promesonotal furrow from the rest of the alitrunk. Prosternum and katapisternum only slightly protruded (less than in *L. plutonia*). In lateral view, upper margin of pronotum almost straight. Mesepinotum also subovoidal, but clearly longer than wide with sinuous sides and more stout than in the other 3 species; its dorsal margin is straight in lateral view, without depression between the mesonotum and the propodeum. Hind dorsal margin of propodeum forming an angle just above the propodeal spiracle. Legs stout, with fore tibiae and all femora less swollen than in *L. zaballosi* and *L. charonea*, but more than in *L. plutonia*. Basitarsus of fore legs without projection on its ventral anterior margin. Tibial spurs as in *L. zaballosi*.

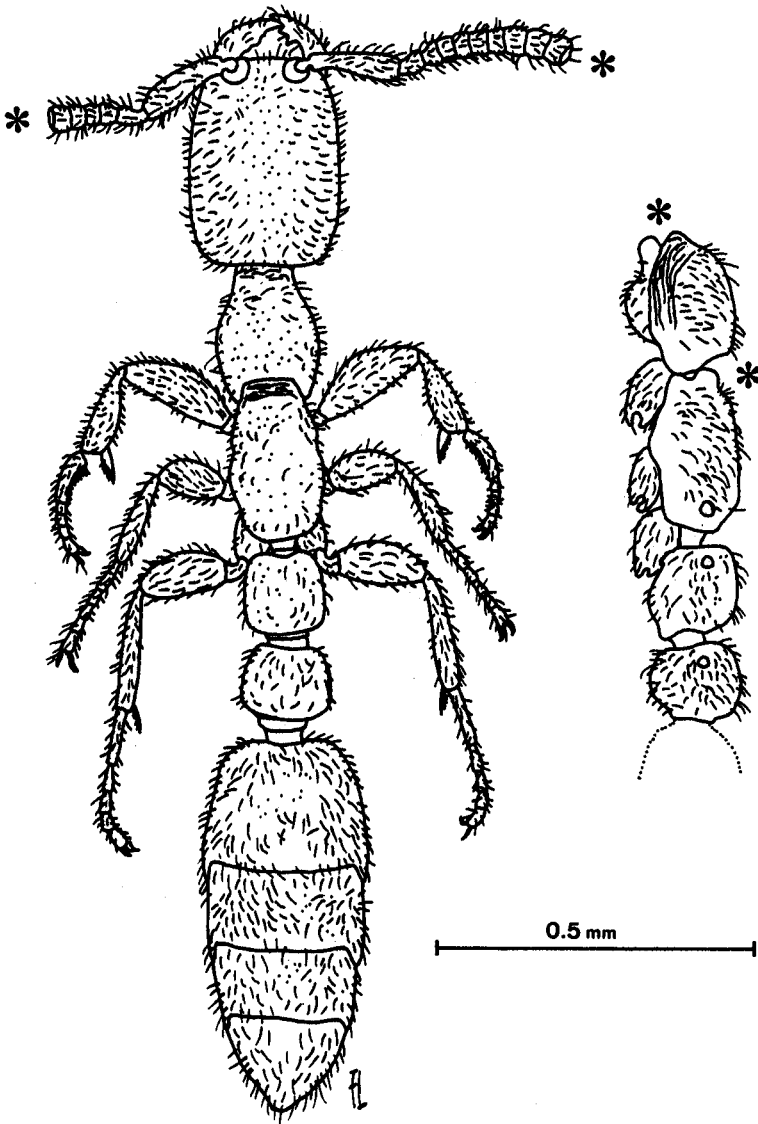


Fig. 9. *Leptanilla ortunoi* n. sp. Holotype worker. Left: dorsal view. Right: lateral view of thorax and petiolar nodes. The asterisks indicate small disjoints between the thoracic sclerites or the lack of terminal antennal segments.

In dorsal view, both petiolar and postpetiolar nodes quadrangular, clearly more stout than in the other 3 species (almost as wide as the propodeum), with their anterior margin feebly emarginated; only the postpetiolar node is feebly wider posteriorly. In lateral view, both nodes dorsally and ventrally convex, although the dorsal margin of the petiolar node is almost straight. Petiolar spiracles clearly visible and comparatively bigger than in the other three species. Dorsal anterior margin of the petiolar node angled, not directed downwards, ventrally with a very small serrated keel constituted by a group of minute teeth. Postpetiolar node feebly emarginated in its anterior margin (clearly different from the other three species), without any projection in its ventral margin.

Gaster ovoidal, more slender than in *L. plutonia*; anterior margin clearly emarginated (more strongly than in the other three species). First gaster tergite longer than the others. Sting long, not protruding, with the bulb clearly wider than the shaft.

Measurements (in mm) and indexes. CL = 0.30; CW = 0.23; SL = 0.12; PL = 0.12; PW = 0.12; PPL = 0.11; PPW = 0.14; TL = 1.62; CI = 79.17; SI = 190.00; PI = 100.00; PPI = 122.22; PPPI = 90.91 (N = 1).

Holotype. Worker from Mirador de García-Aldave, Ceuta, Spain, UTM 30STE87, altitude = 0m, 24-I-1989, V. Ortuño leg. Collected from a moist soil sample with *Asphodellus* roots. Preserved in the Cátedra de Entomología collection, Facultad de Biología, Universidad Complutense de Madrid.

Derivatio nominis. This species is dedicated to Vicente M. Ortuño, who collected it, along with other *Leptanilla* samples, and encouraged us in our work.

Morphological affinities of the new species

The results of the morphometric comparisons between the different species of the genus, by means of the Cluster Analysis, are shown in the Fig. 10 dendrogram. According to the traits studied, the species can be grouped in 3 distinct groups (indicated as I, II and III). Generally speaking, these groups include large (I), medium (II), and small (III) sized species. The species from groups I and II have broader head and pronotum in comparison with group III (see figures in Baroni Urbani 1977). Group I includes *L. escheri*, *L. besucheti*, *L. kubotai* (from South-East Asia and Japan), *L. vaucheri*, and *L. theryi* (from the Mediterranean region). *L. plutonia* n. sp. and *L. ortunoi* n. sp. are also included in this group. Group II includes *L. thai*, *L. havilandi*, *L. butteli*, *L. buddhista*, *L. japonica*, and *L. tanakai* (from South-East Asia and Japan). Group III includes *L. boltoni* (from central Africa), *L. swani*

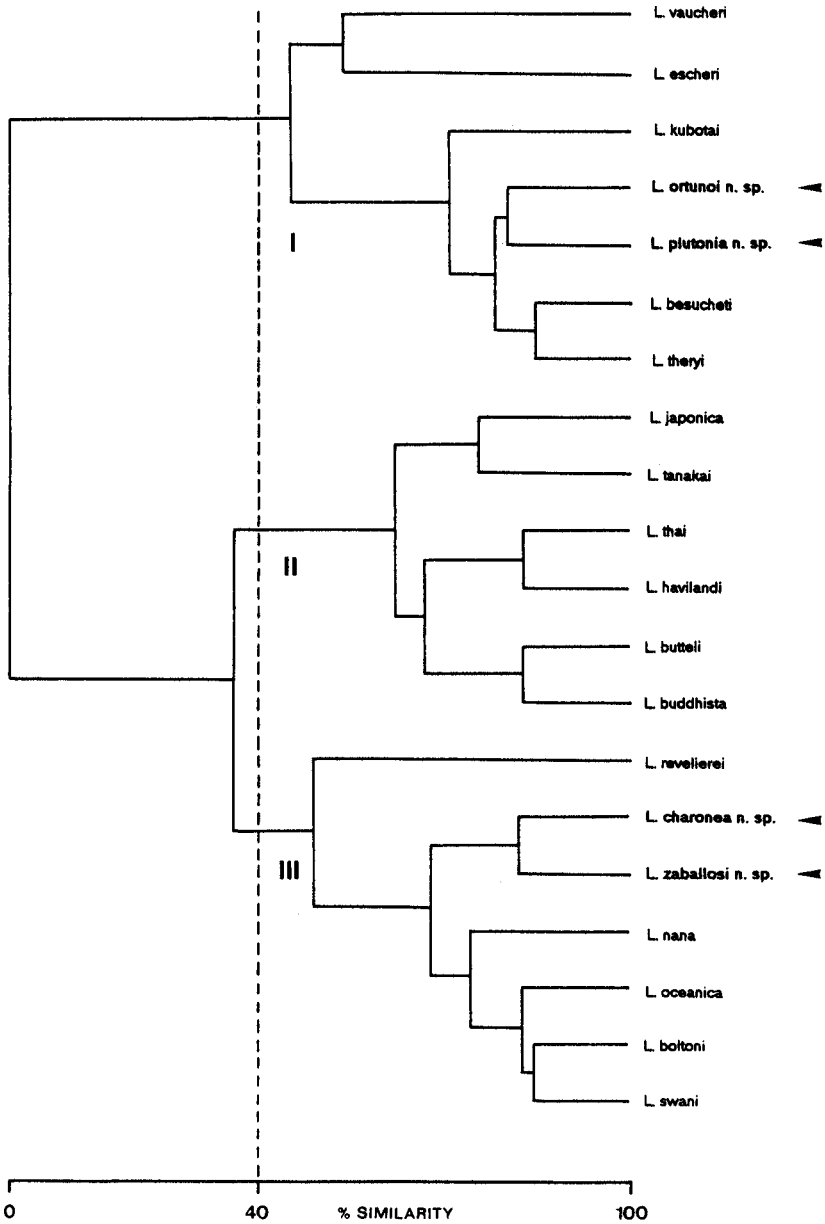


Fig. 10. Results of the Cluster Analysis performed with several morphometric measurements from workers of different *Leptanilla* species. It shows the ordination of most of the known worker-based species of the genus *Leptanilla*. I, II, and III = Ordination groups distinguished at a level of 40 % similarity. The arrows indicate the four new species described in this paper.

(from Australia), *L. oceanica* (from Japan), *L. revelierei*, and *L. nana* (from the Mediterranean region). This last group also includes *L. zaballoi* n. sp. and *L. charonea* n. sp. Therefore, there is no clear geographical grouping in this arrangement.

Our qualitative morphological comparisons between the 4 new species and the rest of the species of the genus (using the type material, as well as the figures and descriptions offered by Baroni Urbani (1977)) corroborate these quantitative ordination results. The four new species are grouped in 2 pairs (*L. plutonia* - *L. ortunoi* and *L. zaballoi* - *L. charonea*) with maximum similarity, within each of the groups in which they are included (Fig. 10). Since the main differences among the new species have been highlighted in their descriptions, we now focus the morphological analysis on the other species of the genus. We have made detailed morphological comparisons between each pair of new species and their nearest Mediterranean species, which were the most difficult taxa to discriminate (some of the most outstanding traits employed for the discrimination of the species are summarized in Fig. 11).

The nearest Mediterranean species to *L. plutonia* and *L. ortunoi* is *L. theryi* (Fig. 10). The rest of the species of this group are easily distinguished from these two new species by the shape of the pronotum in dorsal view (less globular in the new species) and the ventral protuberances of the postpetiolar node in lateral view (which are lacking in the new species). The main traits that differentiate *L. theryi* from *L. plutonia*/*L. ortunoi* are (Fig. 11): (i) tooth-like protuberances of the tentorium thin and pedunculated, (ii) one large apical setae in the inner part of each mandible, (iii) deeper promesonotal furrow, (iv) basitarsus of fore legs with a projection on its ventral anterior margin, (v) katapisternum less protruding, (vi) petiolar and postpetiolar nodes more roundish and with its anterior margin not emarginated, (vii) dorsal anterior margin of the petiolar node more roundish in lateral view, (viii) no ventral keel in the petiolar node, (ix) anterior dorsal and posterior ventral margins of prothorax, and ventral part of petiole stem with a row of minute teeth, (x) pilosity much more scarce.

The only Mediterranean species within the group III are *L. nana* and *L. revelierei*. These are the closer species to *L. zaballoi* and *L. charonea* (Fig. 10). As in the precedent case, the rest of the species of the group can be easily discriminated from the 2 new species by the ventral protuberances of the postpetiolar node in lateral view (which are lacking in the new species). The main traits that differentiate *L. nana* from *L. zaballoi* / *L. charonea* are (Fig. 11): (i) Clypeus protruding, (ii) protuberances of the tentorium not tooth-like, but small and sharp,

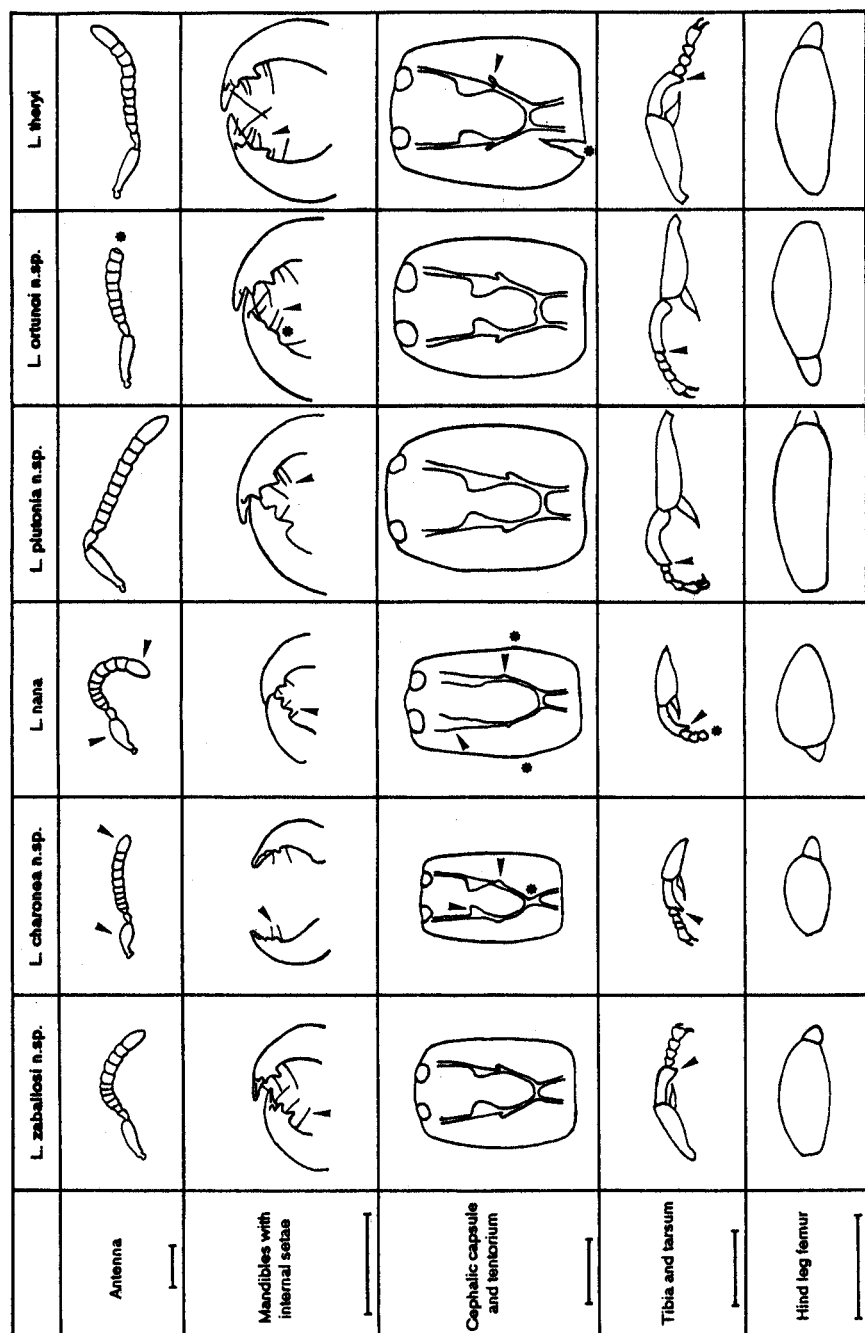


Fig. 11. Comparative table of traits between the four new described species and the type material of *Leptanilla nana* and *L. theryi*. Pilosity omitted. Arrows indicate traits of special interest. The asterisks indicate lack of joints, deformities or broken parts. The lines in the first column represent a scale of 0.1 mm.

(iii) internal plates of the tentorium not roundish, but extended anteriorly, (iv) projection of the basitarsus more protruding, (v) shape of hind leg femur intermediate between *L. zaballoi* and *L. charonea*, (vi) posterior margin of epinotum more angled, (vii) no ventral keel in the petiolar node, (viii) ventral part of petiole stem and petiole node with a row of minute teeth, (ix) postpetiolar node not emarginated in lateral view, (x) pilosity much more scarce.

All the studied specimens of workers of *L. revelierei* (which constitute the majority of the known specimens of the species) are dry-mounted. As commented in the materials and methods section, this kind of preparation makes the study of many traits of internal and external morphology very difficult. The external morphology of these specimens seems to differ from that of *L. charonea* in the same way as *L. zaballoi*. Alternatively, this latter species cannot be properly compared with the type material of *L. revelierei*. We therefore tried to complement the comparisons using the queen morphology and a morphometric analysis of the three species.

The only known female specimen of *L. revelierei* (gynotype) is mounted on a microscope slide, but is in a precarious state (Fig. 12). In spite of some deformities and the lack of some antennal and tarsal segments, several traits, clearly appreciable, differentiate the female of *L. revelierei* from that of *L. charonea* (Figs. 7 & 12): (i) slender scapes, (ii) femora not swollen, (iii) petiolar node roundish in dorsal view, (iv) much larger first gastral tergite and with a semicircular shape.

On the other hand, the results of the Discriminant Analysis (see materials and methods section) show a clear separation between the three species (Fig. 13), and also support the validity of each one. The percentages of well classified individuals are: 100% (*L. charonea*), 90% (*L. zaballoi*), and 87.5% (*L. revelierei*). Only 5 out of 79 specimens were classified by the analysis in a different group (species) from that in which they were previously assigned. Furthermore, all of these badly classified individuals belonged to samples in which the rest of the specimens were well classified, thus indicating that the misclassification was due to individual variability and not to a wrong assignment of a certain sample to a certain species (it can be assumed with a high degree of reliability that all the individuals of a single sample belong to the same colony).

Further observations on outstanding morphological traits:

The fragility and minute size of the *Leptanilla* specimens may lead to mistakes when studying some relevant taxonomical traits of these ants. These should be carefully considered when being studied.

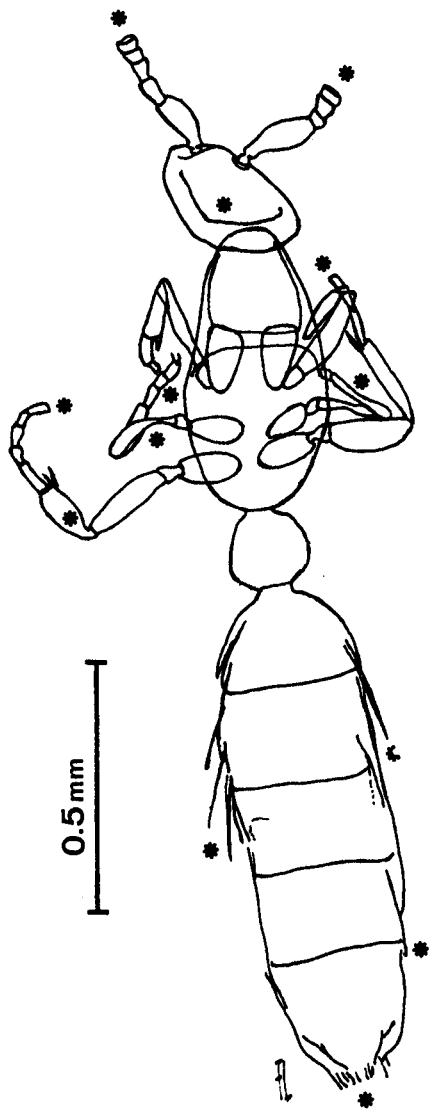


Fig. 12. *Leptanilla revelierei*. Female in dorsal view. Pilosity omitted. Asterisks indicate lack of joints, deformities or broken parts.

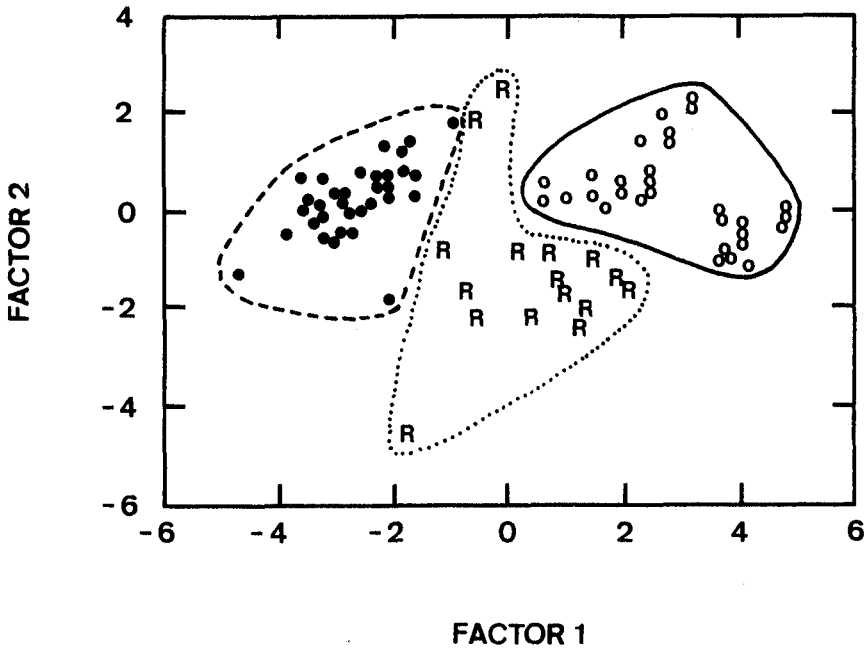


Fig. 13. Results of the Discriminant Analysis using measurements from the head, thorax, and petiole nodes to differentiate *Leptanilla zaballosi* n. sp. and *L. charonea* n. sp. from *L. reveliieri*. White dots = *L. zaballosi* n. sp. Black dots = *L. charonea* n. sp. R = *L. reveliieri*.

On the one hand, some traits vary because of the handling and preservation processes. The colour of the integument might change in time in the preserved specimens. The cleaning process (see material and methods section) produces a slight change (clearing) in the colour, as well. The preparation of the specimens on microscope slides sometimes causes breakages and deformities to different parts of the body. We also noted that the 2 grouped basal teeth of the mandibles may easily break, leaving 2 tiny swellings that could be mistakenly interpreted as blunt teeth. As commented above, the spots on the head are highly variable and cannot be used as a taxonomical trait. The clearing process may also produce a clearing of these spots.

On the other hand, the tibial spurs have been a difficult trait in the worker morphology since the description of the genus. In the diagnosis of *Leptanilla*, Emery (1910) mentioned a single spur in the mid and hind tibiae. Wheeler & Wheeler (1930) later offered a formal definition of the *Leptanillinae* subfamily in which they included the 'tibiae with a pectinate spur' as a trait of the workers. Kutter (1948) described a new genus (*Leptomesites*), whose discrimination was partly based on the presence of two spurs on both tibiae. This genus was later

synonymized with *Leptanilla* by Baroni Urbani (1977) upon considering one of the spurs as a misinterpretation of an agglutination of long setae in the dry specimens. In a more recent work, Kugler (1986) described a new species (*L. judaica*) as having one large pectinated spur and, in some specimens, a second, small simple spur on both tibiae. In all the specimens of the four new species described in the present study, as well as in the type specimens preserved in microscope slides (female of *L. revelierei*, workers of *L. theryi* and *L. nana*), we were able to clearly observe one simple spur in the mid tibiae and a similar spur, plus a second one, large and pectinated, in the hind tibiae. The two spurs of hind legs can only be clearly appreciated in the specimens mounted on slides (under large microscope magnification) and preferably in lateral view. Both cannot be focused simultaneously, and the pectinated spur usually keeps the simple one out of sight. We think that the careful examination of this trait in the workers of other species could lead to the rectification of the diagnosis of the genus and to the elucidation of its taxonomic significance. It must be stressed that the males of all the species of *Leptanilla* have a tibial spur formula of 1:1:2 (i.e., the same as all the workers we have studied), while the males of other genera of the subfamily (as *Noonilla*, *Phaulomyrma* and *Yavnella*) have a formula of 1:2:2 (Wheeler & Wheeler 1930; Taylor 1965; Petersen 1968; Kugler 1986).

DISCUSSION

Until now, only 18 *Leptanilla* worker-based species have been known in the world. In this study we have described 4 new species of the genus, which represents a significant contribution to the taxonomic knowledge of the group. In addition, the female of one of the species has also been described (this caste is known only in 5 species).

The new species were found in a very small geographical area (Fig. 14), which indicates that the genus could be much more diverse (in terms of number of species) than formerly believed. Two of the species are described with an unusually large number of specimens from many samples (40 and 388 workers of *L. charonea* and *L. zaballoi*, respectively). This has allowed the variability of some traits to be studied. The other two new species are only described with a single specimen. Although this might be hazardous, we finally decided to describe them for several reasons. First, the unequivocal distinction from the other species of the genus. Second, the unforeseeability of finding new specimens after several years of searching. Finally, the importance of presenting new taxa of a badly known and interesting group from the point of view of phylogeny. In fact, 7 male-based species have been described with a single specimen, 4 worker-based species with 2 or 3 specimens (Baroni Urbani 1977), and even a new genus with a single

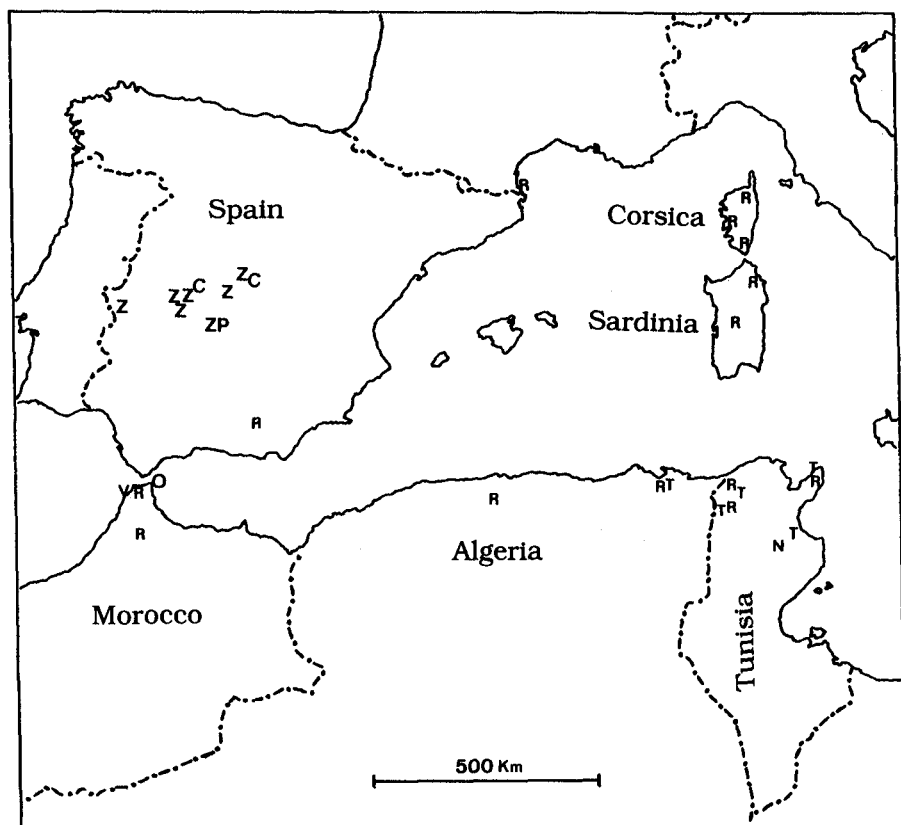


Fig. 14. Distribution map of the worker-based species of *Leptanilla* from the Western Mediterranean region. C = *Leptanilla charonea* n. sp. N = *L. nana*. O = *L. ortuno* n. sp. P = *L. plutonia* n. sp. R = *L. revelierei*. T = *L. theryi*. V = *L. vaucheri*. Z = *L. zaballosi* n. sp.

female (Bolton 1990).

In one case, 2 of the new species (*L. zaballosi* and *L. charonea*) were found in the same place (samples from Casa de Campo, Madrid). In spite of the rarity of the findings of these insects, this is not infrequent. The four male-based Mediterranean species of *Leptanilla*, for example, were found in the same locality (Kairouan, Tunisia) (Santschi 1907, 1908). There are also examples of this kind of simultaneous finding (several species of the same genus in the same place) in other hypogaean insects, such as some beetles of the families Pselaphidae and Trechidae (Outerelo 1980; Ortuño 1988; Zaballos 1989). This emphasizes the caution that should be taken when considering the geographical distribution of a species as a taxonomical criterion.

The ordination of the worker-based species of *Leptanilla* obtained through some morphometric traits corroborates the observations of Baroni Urbani (1977) on the lack of clear relationships between geography and morphology in the genus. The qualitative morphological study of the specimens of the new species and the type material are in accordance with this conclusion. The scarcity of specimens and the large information gaps in the distribution makes it quite difficult to analyze this problem more deeply.

The genus *Leptanilla* constitutes a taxonomic group with workers with a great similarity between species. Baroni Urbani (1977) has postulated that this could be due to strong selective pressure because of their hypogaecic life. For example, the dorsoventral flattening of the body of the workers is probably adaptive for the movement of the ants through the interstices of the soil (Masuko 1990). The new morphological traits proposed in this paper seem to be useful for the comparative study and identification of the species. Within the new species described, for example, *L. zaballoi* and *L. charonea* are very similar in many aspects of their external morphology, but can be clearly discriminated by using traits such as the morphology of the tentorium, katepisternum, femora, basitarsus of fore legs, and mandibular setae. These traits have been shown to be also effective for the distinction between other species of the genus. Most of them are much more easily appreciable and require less sophisticated preparations than other frequently used discriminant traits such as teeth or palps.

The abundance of *Leptanilla* findings seems to be due to the biotopes and collection techniques used. These small ants have been considered as hypogaecic since their discovering (Emery 1870, 1875). They have been found in some occasions beneath stones (Emery 1875; Baroni Urbani 1977; Kugler 1986; Tinaut 1987) or in litter samples (Yasumatsu 1960; Baroni Urbani 1977; Bolton 1990), but these findings are probably fortuitous. In more than a century of entomological surveys on soil arthropods all over the world since the first *Leptanilla* was discovered, only a few scattered samples of this genus have been collected. Baroni Urbani (1977) commented that many areas of the Mediterranean region and India, in which the presence of Leptanillinae is predictable, have been unsuccessfully surveyed by numerous researchers of hypogaecic fauna. He concluded that the finding of these ants is unpredictable. However, the collection methods and biotopes are not specified. The 'lavage de terre' collection method (Normand 1911) has proven to be successful for the capture of different groups of 'rare' hypogaecic insects. Santschi (1915) found specimens of *L. nana* and *L. theryi* in Tunisia using this method. More recently, many

new species of hypogaecic beetles have been discovered on the Iberian Peninsula by the same procedure (Outerelo 1980; Outerelo & Gamarra 1986; Ortuño 1988; Hernán & Outerelo 1989; Zaballo 1989). It is believed that the success of this method is due to the removal and processing of a large quantity of soil, thus increasing the chances of finding specimens of these hypogaecic insects, whose populations are likely very small. In fact, few specimens (of non-social insects) can usually be collected from each sample. The large samples of *Leptanilla* found are easily attributable to the capture of almost complete colonies. The washing and filtering of the samples, however, is not essential, since samples taken directly to the laboratory and separated in Berlese-Tullgren devices are likewise effective (V. M. Ortuño, pers. com.). The type of biotope surveyed is probably more important than the collection method. As well as all of the species of beetles mentioned above, most of the *Leptanilla* samples come from small slopes of seasonal water courses, which are poor in organic content but have a high degree of moisture. The species richness of hypogaecic insects on these slopes was discovered by chance and has been confirmed by repeated findings during recent years (J. P. Zaballo, pers. com.). Since the beginning of the intensive collection surveys in these kind of biotopes (1988-1991), samples containing *Leptanilla* specimens have appeared regularly.

The ultimate causes of the appearance of hypogaecic insects in these biotopes are, however, difficult to ascertain. The samples with *Leptanilla* specimens were taken from different types of soils (arkose, clay, gypsum, slate, limestone) and from sites with different types of vegetation cover (pastures, Holm oaks, pines). Therefore, these environmental factors do not seem to play an important role for the presence of these insects. On the other hand, all the surveyed slopes had in common a high degree of moisture. The water content was also high in the places where the samples that did not come from slopes were taken.

Two possible hypothesis can be outlined for the explanation of the richness of the described slopes. (1) They constitute a peculiar biotope because of some unknown physical feature related to their structure as slopes. As a comparison, some physical peculiarities of the soil according to the depth have led to the description of new subterranean biotopes (Juberthie *et al.* 1980). (2) They represent a means of access to a deep part of the soil, which is not usually - or is hardly - surveyed when excavating directly from the surface. On behalf of this second hypothesis we have repeatedly found the evidence that the colonies of *Leptanilla* are collected close to the soil surface (in different places,

which are not slopes) only when there is a high degree of moisture. Both the *L. charonea* colony that we found by excavation and all the colonies collected by Masuko (1990) using the same procedure were collected in such conditions. Furthermore, Wheeler (1932) mentioned that the workers and females of *Leptanilla* 'come to the surface of the soil only under unusual conditions, such as excessive rainfall'. These exceptional external conditions could eventually reproduce usual conditions of a greater depth in the soil. It is evident that these matters require further study and must be analyzed specifically; their solution would be of great interest for the ecology of hypogaeic insects.

Given the relatively high frequency of appearance of *Leptanilla* in all the areas surveyed by the methods and in the biotopes described, we believe that these minute ants could be much more frequent and widely distributed geographically than what was believed until now. A search guided by these criteria will probably lead to the discovering of new species of this badly known group.

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